PRE AMENDMENT A
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PATENT
Our Docket: P-IX 4102

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Huse and Glaser

Serial No.: Herewith

Filed: Herewith

For: ANTI-$\alpha_v\beta_3$ RECOMBINANT HUMAN ANTIBODIES, NUCLEIC ACIDS ENCODING SAME AND METHODS OF USE

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Sir:

Entry of the amendments below and consideration of the following remarks is respectfully requested.

PRELIMINARY AMENDMENT

Please amend the title page as follows:

Please delete the title starting at line 5 and insert therefore:

COMPOSITIONS AND METHODS FOR PRODUCING ENHANCED ANTIBODIES

Please amend the specification as follows:

On page 1, please delete the title starting at line 1, and insert therefore:

COMPOSITIONS AND METHODS FOR PRODUCING ENHANCED ANTIBODIES

On page 14, please delete footnote 2 starting at line 11 and ending at line 12, and insert therefore:

[2] Residue numbering follows the nomenclature of Chothia et al., *supra*

On page 16, please delete the paragraph starting at line 10 and insert therefore:

As used herein, the term "functional fragment" when used in reference to Vitaxin, to a LM609 grafted antibody or to heavy or light chain polypeptides thereof is intended to refer to a portion of Vitaxin or a LM609 grafted antibody including heavy or light chain polypeptides which still retains some or all of the $\alpha_v\beta_3$ binding activity, $\alpha_v\beta_3$ binding specificity and/or integrin $\alpha_v\beta_3$-inhibitory activity. Such functional fragments can include, for example, antibody functional fragments such as Fab, $F(ab)_2$, Fv, single chain Fv (scFv). Other functional fragments can include, for example, heavy or light chain polypeptides, variable region polypeptides or CDR polypeptides or portions thereof so long as such functional fragments retain binding activity, specificity or inhibitory activity. The term is also intended to include polypeptides encompassing, for example, modified forms of naturally occurring amino acids such as

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D-stereoisomers, non-naturally occurring amino acids, amino acid analogues and mimetics so long as such polypeptides retain functional activity as defined above.

On page 64, please delete the paragraph starting on line 1, and insert therefore:

Grafted LM609 heavy and light chain V regions were constructed by mixing 5 overlapping oligonucleotides at equimolar concentrations, in the presence of annealing PCR primers. The heavy chain oligonucleotides map to the following nucleotide positions: V_H oligonucleotide 1 (V_H oligo1), nucleotides (nt) 1-84; (SEQ ID NO:9); V_H oligo2, nt 70-153, (SEQ ID NO:10); V_H oligo3, nt 139-225 (SEQ ID NO:11); V_H oligo4, nt 211-291 (SEQ ID NO:12); V_H oligo5, nt 277-351 (SEQ ID NO:13). Similarly, the Vitaxin light chain oligonucleotides map to the following nucleotide positions: V_L oligonucleotide 1 (V_L oligo1), nucleotides (nt) 1-87; (SEQ ID NO:14); V_L oligo2, nt 73-144, (SEQ ID NO:15); V_L oligo3, nt 130-213 (SEQ ID NO:16); V_L oligo4, nt 199-279 (SEQ ID NO:17); V_L oligo5, nt 265-321 (SEQ ID NO:18). The nucleotide sequences of oligonucleotides used to construct grafted LM609 heavy and light chain variable regions are shown in Table 6. Codon positions 49 and 87 in V_L oligo3, and V_L oligo4 represent the randomized codons. The annealing primers contained at least 18 nucleotide residues complementary to vector sequences for efficient annealing of the amplified V region product to the single-stranded vector. The annealed mixture was fully converted to a double-stranded molecule with T4 DNA polymerase plus dNTPs and ligated with T4 ligase.

On page 83, through 84, please delete the paragraph starting at page 83, line 27, and insert therefore:

Oligonucleotides encoding a single mutation were synthesized by introducing NN(G/T) at each CDR position as described previously (Glaser et al., *supra*). The antibody libraries were constructed in M131XL604 vector by hybridization mutagenesis as described previously, with some modifications (Rosok et al., J. Biol. Chem. 271:22611-22618 (1996); Huse et al., J. Immunol. 149:3914-3920 (1992); Kunkel, Proc. Natl. Acad. Sci. USA 82:488-492 (1985); Kunkel et al., Methods Enzymol. 154:367-382 (1987)). Briefly, the oligonucleotides were annealed at a 20:1 molar ratio to uridinylated LM609 grafted antibody template (from which the corresponding CDR had been deleted) by denaturing at 85°C for 5 min, ramping to 55°C for 1 h, holding at 55°C for 5 min, then chilling on ice. The reaction was extended by polymerization and electroporated into DH10B and titered onto a lawn of XL-1 Blue. The libraries consisted of pools of variants, each clone containing a single amino acid alteration in one of the CDR positions. Utilizing codon-based mutagenesis, every position in all of the CDRs was mutated, one at a time, resulting in the subsequent expression of all twenty amino acids at each CDR residue (Glaser et al., *supra*). The CDR libraries ranged in size from 288 (L3) to 416 (L1) unique members and contained a total of 2336 variants.

On page 87, please delete the title to the Table starting at line 8, and insert therefore:

Table 8: Capture Lift Screening of LM609 grafted antibody CDR Libraries.

On page 87, please delete footnote 1, starting at line 18, and ending at line 20, and insert therefore:

[1]Number of unique clones based on DNA sequence. Thirty-two codons are used to express all twenty amino acids at each position.

On page 97, please delete Table 10, and insert therefore:

Table 10: Identification of Optimal Combinatorial Mutations

		sequence†									
library*	clone	L1 32	L3 92	L3 96	H2 60	H3 97	H3 101	H3 102	k_{on} (x10^4) ($M^{-1}s^{-1}$)	k_{off} (x10^{-3}) (s^{-1})	Kd (nM)
wild type		H	G	H	L	Y	A	Y	18.0	4.97	27.6
F32	17	F						S	25.1	0.138	0.5
	7	F			P	H		S	20.4	0.236	1.2
	56	F			P			S	26.6	0.135	0.5
	C59	F			P			D	26.5	0.137	0.5
	C176	F			P			T	22.5	0.192	0.9
	V357D	F						D	27.9	0.140	0.5
N92	C119		N		P			S	21.5	0.316	1.5
L96	8F9			L	P	H		S	47.5	0.280	0.6
	C29			L	P	H	Y	S	67.5	0.343	0.5
	2G4			L				S	60.3	0.229	0.4
	6H6			L		H		S	50.4	0.187	0.4
	C37			L			Y	E	44.8	0.147	0.3
	6D1			L	P		Y	S	41.0	0.158	0.4
	6G1			L	P			S	38.9	0.280	0.7

Please insert new pages 101 through 130 and renumber original pages 101 through 116 as pages 131 through 146, respectively.

Please amend the claims as follows:

Cancel claim 1 without prejudice. Add new claims 105-117 as follows:

80. A grafted antibody, or functional fragment thereof, comprising an association rate constant (k_{on}) greater than 1.4 X 10^6 M^{-1} sec^{-1}.

81. The grafted antibody, or functional fragment thereof, of claim 80 further comprising an association constant (K_a) greater than 5 X 10^9 M^{-1}.

82. The grafted antibody, or functional fragment thereof, of claim 80, wherein said k_{on} is greater than 2.7 X 10^6 M^{-1} sec^{-1}.

83. The grafted antibody, or functional fragment thereof, of claim 82 further comprising an association constant (K_a) greater than 1.0 X 10^{10} M^{-1}.

84. The grafted antibody, or functional fragment thereof, of claim 80, comprising a humanized antibody, or functional fragment thereof.

85. A method for producing an enhanced antibody, or functional fragment thereof, comprising:

(a) modifying a parent antibody, or functional fragment thereof;

(b) obtaining one or more variant antibodies, or functional fragments thereof, said one or more variant antibodies, or functional fragments thereof, comprising one or more amino acid substitutions in one or more variable regions compared to said parent antibody, and

(c) measuring the association rate constant (k_{on}) of said one or more variant antibodies, or functional fragments thereof, to an antigen, wherein a variant antibody, or functional fragment thereof, having an association rate to an antigen that is 4-fold higher or greater compared to the rate of said parent antibody binding to said antigen is an enhanced antibody, or functional fragment thereof.

86. The method of claim 85, further comprising isolating said enhanced antibody, or functional fragment thereof.

87. The method of claim 85, wherein said one or more amino acid substitutions are in one or more CDRs.

88. The method of claim 85, wherein said one or more amino acid substitutions are in one or more framework regions.

89. The method of claim 85, wherein said amino acid substitutions are in one or more CDRs and one or more framework regions.